QTLs Detection for Growth and Initial Latex Production in Rubber (*Hevea brasiliensis*)

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Abstract

The objective of this study was to analyse the genetic determinism of growth and latex production in rubber tree by a QTL approach. The plant material consisted of 196 progenies derived from the F1 family RRIM600 x PB217. A genetic linkage map was built for this family with 229 SSR markers (microsatellites) and 198 AFLP markers. Phenotyping was carried out over a 6-year period on a field trial of 5 hectares, with around 2400 trees measured individually. A high broad sense heritability was found for latex production, and a major QTL directly associated to production was discovered (Hbg16a131). This QTL explained from 32 % to 59 % of the genetic variance of the production traits. It was also associated to other traits that were strongly correlated to production such as inorganic phosphorus and dry rubber content (latex diagnostic), as well as plugging index. This finding indicates the existence of one major gene (or a cluster of genes) located on linkage group 16 and involved in the genetic determinism of latex production. A second important QTL associated to the girth of the trunk (growth) was detected (Hbg3a312). Depending on the age of the trees, it explained from 16 % to 28 % of the genetic variance of the girth. By contrast, no significant QTL was found for sucrose content of the latex, as estimated independently from the production, so suggesting that sucrose genetic determinism might not include any single gene having by itself some important effect. The application of one ethephon stimulation did not evidence any specific QTL. Those results pave the way for Markers-Assisted Selection (MAS) as applied to clonal selection in rubber. Genotyping the candidates to selection for the 2 QTLs with important effects should allow a much more accurate estimation of genetic values as soon as at the first selection stage.

Key words

Rubber tree (*Hevea brasiliensis*), Natural rubber, Growth, Latex Production, Latex diagnostic, Plugging index, Ethephon stimulation, Genetic linkage map, Heritability, Genetic correlations, Quantitative Trait Loci (QTLs), Marker-Assisted Selection.

Introduction

Over the 13,300 million hectares (Mha) of emerged lands on the Earth, agriculture covers 1,500 Mha, including 150 Mha of permanent crops and 10 Mha of rubber that is grown predominantly in South-East Asia (92 %) by smallholders (80 %). Thailand is the first natural rubber producing country. Since 30 years, the share of natural rubber in the total amount of elastomers (natural and synthetic) was increased from 30 % to 43 % mainly due to economic growth and increasing demand in Asia. Due to the constraints of land use by agriculture, and the need for developing food crops and protecting natural ecosystems, increasing latex production cannot rely only on the extension to new areas. Therefore there is an economic interest for increasing land and labour productivity in rubber cropping with new varieties.

Currently, rubber varieties are highly heterozygous clones multiplied by budding onto seedling rootstocks. Recombination between parental clones by hand pollination generates full-sib families of seedlings that are submitted to initial screening in a Seedling Evaluation Trial (SET). The selected genotypes are then budded and passed to two successive selection trials: the Small Scale Clonal Trial (SSCT) and the Large Scale Clonal Trial (LSCT). The first selection stage (SET) is considered as the weak point of the process, as far as it is applied to a very large population of genotypes with poor information available. New tools able to provide more accurate genetic information would improve the efficiency of that initial selection stage (Clément-Demange et al., 2007).

Following the traditional agromorphological markers, proteic and DNA genetic markers have known fast development since 30 years, with isozymes, RFLP (Restriction Fragment Length Polymorphism), and then with targeted PCR techniques (Polymerase Chain Reaction), especially SSR markers (Single Sequence Repeats also called microsatellites) that have a high level of polymorphism. Those genetic markers have been used, in rubber as in many other plants, for the description of the neutral genetic diversity of genetic resources, and the identification of cultivars and of their parentages (Seguin et al., 2003). With the identification of an increasing number of molecular genetic markers, mapping of loci involved in quantitative variation, i.e., quantitative trait loci (QTL; Geldermann 1975), has progressively become a central method in quantitative genetics.

QTL detection is typically based on joint genotyping and phenotyping of a segregating population. Due to the diversity of its alleles (polymorphism), each marker makes possible the partition of a population of progenies in genotypic classes. For any measured quantitative trait, it is possible to assess the significance of the differences between those classes and to identify genetic associations due to linkage desequilibrium between the marker and the genetic factor (genes or clusters of genes) underlying the expression of the trait. Moreover mapping of the markers makes possible the approximate localization of the involved genetic factors on the genetic map. Although molecular genetic markers are non-expressed DNA fragments (neutral or anonymous markers), as opposed to ESTs (Expressed Sequence Tags) or full-length gene sequences, QTL detection is seen as a way to investigate the number of genes that control quantitative traits, the magnitude and the distribution of their effects. As a result, the markers associated to the QTLs can become new sources of genetic information in the framework of Markers-Assisted Selection (MAS).

The first genetic linkage map of rubber was developed at Cirad, based on the F1 cross PB260 x RO38, initially with RFLP markers (Lespinasse et al., 2000a) and it was successfully applied to the identification of QTLs associated to genetic resistance to South American Leaf Blight (Lespinasse et al., 2000b; Le Guen et al., 2003; Le Guen et al., 2007). Based on this experience, a new QTL approach was developed by use of the 2 Wickham parents of the F1 family RRIM600 x PB217, for studying the genetic determinism of growth and latex production (Rattanawong, 2006).

Material and methods

Physiological classification of rubber clones

Latex production is mainly determined by the physiological classification of the clones for latex cells functioning, developed through "Latex Diagnostic" measurements. Dry rubber content (Drc, %) is an indicator of the viscosity of the latex that influences the duration of latex flow. Sucrose content (Suc, in millimolar mM) is an indicator of the balance between the influx of sucrose into the latex cells and its consumption for energy production (glycolysis) and isoprene anabolism. Inorganic phosphorus content (Pi, mM) is an indicator of the intensity of energetic metabolism. Thiol content (Rsh, mM) is an approximate indicator of the tolerance of the cells to oxidative stress and abiotic stress (Jacob et al., 1987). This classification makes possible to predict the response of clones to tapping intensity and to ethephon stimulation (2-chloro-ethyl phosphonic acid). As a matter of fact, ethylene is known as a major plant hormone specialized in the response to biotic and abiotic stress.

Plant material

The two heterozygous parents RRIM600 and PB217 were chosen for their contrasted positions in the physiological classification of rubber clones. As most clones, RRIM600 has a rather intensive metabolism, whereas PB217 latex cells have a low initial metabolism but a high sucrose content fitted to the intensification of the tapping and stimulation system (Gohet et al., 2003).

The F1 family RRIM600 x PB217 was created by hand pollination in 2000. The genetic linkage map (genotyping) was developed over 18 working months for one person. A field trial (phenotyping) was planted in June 2002 with 196 progenies in the form of budded trees, and measurements were carried out since then.

The genetic linkage map

Hand pollination generated more than 600 progenies in the family RRIM600 x PB217. For a number of 427 molecular genetic markers (229 SSR and 198 AFLP markers), genotyping was achieved for the 196 progenies that were planted in the field trial. The genetic linkage map was built by the double pseudo-test cross strategy. The total length of the map, distributed over a number of 18 linkage groups corresponding to the 18 chromosomes of the haploid genome of rubber, was of 2075 cM. The average length between two successive markers varied from 3.14 to 7.12 cM

depending on the linkage group, with a global average of 4.86. The largest length between two successive markers was of 38 cM (Lekawipat 2005; Prapan, 2006). The genotypic data and the positions of the markers on the linkage groups make the basic genetic information to be used jointly with the field phenotypic data for QTL detection.

The field trial

The main characteristics of the field trial are presented in table 1. "Alpha-plan" design, based on incomplete small blocks (Patterson and Williams, 1976) was chosen as fitted to the comparison of a large number of treatments (196 progenies + the two parents) on a rather large and probably heterogeneous field area, with optimized control of the environmental variation. A Cirad software ("Alpha-plan", J.P. Jacquemoud-Collet, unpublished) was used for drawing the randomized field map.

Table 1: Characteristics of the Genmap field trial planted.

Field planting design : square planting, 4 x 4 m
Planting density : 625 trees / ha
Total area (without borderlines) : 5.12 ha
Total number of trees : 3200
Number of budded trees of the same clone per elementary plot : 4
Total number of plots : 800
Number of clones : 200
Number of full replications (blocks) : 4
Number of elementary plots per block : 200
Number of sub-blocks per block : 25
Number of plots per sub-block : 8
Statistical design : « alpha-plan » (incomplete block design)
Sources of variation :
Genetic (between clones) : G
Blocks : B (controlled effect)
Sub-blocks : S (controlled effect)
Error : E (uncontrolled effect)
h^2l = G / (G+E)

The 196 progenies were coded from 1 to 196. Each parent was coded twice, so making 2 different treatments for securing their representation in the trial (RRIM600 coded 197 and 198, and PB217 coded 199 and 200). Therefore there were 200 genetic treatments (budded clones) in the trial. The trial was made of 4 blocks making complete replications of the 200 treatments. Each block was divided into 25 sub-blocks of 8 plots. Each plot representing one clone was made of a linear segment of 4 budded trees (4 blocks x 25 sub-blocks x 8 clones x 4 trees = 3,200 trees).

Thanks to the optimization by "Alpha-plan" software, each clone was set into 4 sub-blocks (one sub-block per block), it was directly compared to 7 other clones in each sub-block and it was directly compared to 7 x 4 = 28 different other clones in the trial. Overall there were (200 x 28) / 2 = 2800 direct comparisons between different couples of clones within the sub-blocks, as compared to a possible maximum number of (200 x 199) / 2 = 19900 couples of clones.

Measurements

Growth

Growth was studied by measuring the girth of the trunk and the height of the trees twice a year, at the beginning of the rainy season and at the beginning of the dry season. Bark depth was measured once at 6 years of age.

Girth was measured at 1-m high as well as at 1.7-m high on around 2400 trees. The average of the 2 data was used for analysis :

- G18: girth at 18 months of age (beginning of dry season)
- G23: girth at 23 months of age (beginning of rainy season)
- G31: girth at 31 months of age (beginning of dry season)
- G36: girth at 36 months of age (beginning of rainy season)
- G43: girth at 43 months of age (beginning of dry season)
- G47: girth at 47 months of age (beginning of rainy season)
- G53: girth at 53 months of age (beginning of dry season)
- G59: girth at 59 months of age (beginning of rainy season and of tapping)
- G64: girth at 64 months of age (beginning of dry season and end of tapping)
- G71: girth at 71 months of age (beginning of rainy season)

Based on those girth measurements, girth increments were calculated:

- P1: G23-G18, 5 months of dry season
- P2: G31-G23, 8 months of rainy season
- P3: G36-G31, 5 months of dry season
- P4: G43-G36, 7 months of rainy season
- P5: G47-G43, 4 months of dry season
- P6: G53-G47, 6 months of rainy season
- P7: G59-G53, 6 months of dry season
- P8: G64-G59, 5 months of tapping under rainy season
- P9: G71-G64, 7 months of dry season
- R21: girth increment P2 + P4 + P6 = 21 months of rainy seasons
- D20: girth increment P1 + P3 + P5 + P7 = 20 months of dry seasons
- D27: = D20 + P9, 27 months of dry seasons.

Heights:

- H17: height at 17 months of age
- H23: height at 23 months of age
- H30: height at 30 months of age
- H36: height at 36 months of age
- H43: height at 43 months of age
- H47: height at 47 months of age
- H53: height at 53 months of age, before the first tapping period
- H67: height at 67 months of age, after the first tapping period.

Bark depth was measured once in 2008 (B61).

Latex production

A first period of tapping was carried out from June to October 2007 on around 2,350 trees with girth at 1-m high higher than 25 cm. Average girth of tapped trees was of 30.9 cm. Tapping was in S/2 d/3 7d/7 with no ethephon stimulation. Due to rain causing the canceling of some tappings, the number of tappings over the period (153 days) was of 44 for replications 1 and 4 (alternance A), and of 42 for replications 2 and 3 (alternance B). A second period of tapping was carried out from June to September 2008 with the same tapping system, and with one stimulation applied in the middle of July (20 mg of ethephon per tree). Average girth of tapped trees was of 37.2 cm. The number of tappings over the period (113 days) was of 24 for replications 1 and 4 (alternance A), and of 26 for replications 2 and 3 (alternance B).

From June to October 2007, the cumulated latex production of each of the five months (P51, P52, P53, P54, P55) was weighed for each individual tree (around 2,350 trees). The cumulated production over the 5 months was calculated (P5cum).

In 2008, cumulated latex production of the six weeks before stimulation was weighed foe each individual tree (P61). After stimulation, a series of individual tappings were weighed, in order to attempt to characterize the evolution of the production:

- P6S1: 1139 trees of replications 1 and 4, 2 days after stimulation
- P6S2: 1210 trees of replications 2 and 3, 3 days after stimulation
- P6S3: 1209 trees of replications 2 and 3, 6 days after stimulation
- P6S4: 1095 trees of replications 2 and 3, 12 days after stimulation
- P6S5: 1144 trees of replications 1 and 4, 14 days after stimulation
- P6S6: 1145 trees of replications 1 and 4, 17 days after stimulation
- P6S7: 1220 trees of replications 2 and 3, 21 days after stimulation.

The cumulated production of those 7 individual tappings was calculated (P6S17cum). The cumulated production of the 10 following tappings was weighed (P6S8). The cumulated production of 2008 and the cumulated production of 2007 and 2008 were calculated (P6cum and Pcum).

Latex Diagnostic and plugging index

Latex Diagnostic (Drc, Suc, Pi, Rsh) was measured individually on nearly 800 trees (4 trees per clone) repeatedly at 5 successive periods along the duration of tapping for characterizing the evolution of latex production (3 times in 2007, and 2 times in 2008, one before stimulation, and the other from 44 to 55 days after stimulation).

On every tapping day, latex production is the result of latex flow from the tapping moment to the coagulation of latex on the tapping-cut a few hours later. Kinetics of latex flow is made of 2 phases, the first one mainly determined by turgor pressure in the latex cell, and the second one due to the influx of water from xylem to latex cells and the latex flow until coagulation on the tapping cut. For characterizing those 2 phases, the Plugging Index method (Milford et al., 1969) consists in measuring the volume or weight of latex flow of the first 5 minutes (W1), and the volume or weight of the ensuing latex flow until coagulation (W2). The Plugging Index is the ratio W1 / (W1 + W2). Plugging Index was measured twice successively in 2007, for two individual tappings, on the same trees also measured for Latex Diagnostic.

Data analysis

SAS statistical software (SAS Institute, 1988) was used for checking the normality of data distributions ("Sas-Insight"). Data distributions were found normal or close to normality for girth, height, bark depth, and Drc measurements. Transformation y = log (x+1) was applied to production traits, Suc and Pi measurements for getting data distributions close to normality. Transformation y = root (x) was applied to Rsh.

Although all measurements were carried out on individual trees, statistical analyses were carried out on the means of the 800 plots. SAS was used for calculating the means per plot ("Means" procedure), for estimating the variance components and the broad sense heritabilities of the traits ("Varcomp" procedure), and for estimating the adjusted means of the genotypes and their associated standard deviations, taking into account the lacking data and varying numbers of data per genotype ("Glm" and "Lsmeans" procedures). Those adjusted means were considered as the estimated genetic values of the clones.

The correlations between the estimated genetic values of the traits for the 200 genotypes were studied by use of STATBOX statistical software (Principal Component Analysis, Pearson correlation coefficients) in order to identify the main independent sources of physiological variation, as it is waited that the same QTL will be found attached to different highly correlated traits. For all the correlation coefficients, degree of freedom = 200-2 = 198, therefore r = 0.14 is significant for α = 0.05, and r = 0.18 is significant for α = 0.01.

"MapQTL5" software (Van Ooijen, 2004) was used for QTL detection, with the basic option "Interval Mapping" fitted to normally distributed data, by use of 3 data files:

- List of the bi-allelic genotypes for each progeny and for each marker
- File of assignation of the markers to their positions on the 18 linkage groups
- File of the quantitative data (estimated genetic values) for each trait and each progeny.

The level of significance of QTL detection is given by the lod-score parameter. The significance threshold level with α = 0.05 was estimated by the "permutation test" option of "MapQTL5". Quantitative data are permuted so that observed QTLs can be due only to random effects. On a series of 1,000 permutations, the probability to identify one QTL genome wide by chance was found less than 5 % for a threshold level of lod-score = 4.5.

Ecological conditions of the field trial

Soil is sandy with fast drainage of excess water during the rainy season. There is a lateritic hard pan at around 50 cm below ground level that impedes the development of taproots. The rainy season spreads from March to October and culminates in September. Dry season (rainfall lower than 50 mm per month) can last from 3 to 5 months depending on the year. Average annual rainfall over the last 20 years was of 1294 mm, with a minimum of 1062 mm in 2004. Therefore water availability can be considered as limiting for rubber cropping in this area.

Results

Range of the estimated genetic values of the clones

Table 2 provides the minimum, maximum and average of the 200 estimated genetic values of the progenies and parents for the main traits: girth G59, height H53, girth increments in rainy and dry seasons R21 and D20, bark depth B61, productions P5cum, P61, P6S17cum, P6S8, P6cum, and Pcum, traits Drc, Suc, Pi, and Rsh of the average of 5 series of Latex Diagnostic, and Plugging Index. The increase in latex production from P61 to P6S17cum and P6S8 is due to stimulation.

Table 2: Minimum, maximum and average of the estimated genetic values of the clones for the main traits measured in the trial.

	G59	**H53**	**R21**	**D20**	**B61**
	cm	m	cm	cm	Mm
Min	25.9	6.07	11.59	1.12	4.33
Max	35.9	9.26	20.23	8.09	6.55
Average	30.7	7.80	16.37	4.43	5.51

	P5cum	**P61**	**P6S17cum**	**P6S8**	**P6cum**	**Pcum**
	g/t/t	g/t/t	g/t/t	g/t/t	g/t/t	g/t/t
Min	1.8	2.3	6.8	6.1	6.0	3.2
Max	18.3	39.6	65.0	52.9	47.0	26.0
Average	7.0	12.2	20.7	19.8	18.9	11.1

	Drc	**Suc**	**Pi**	**Rsh**	**Pind**
	%	mM	mM	mM	%
Min	29.7	9.2	6.3	0.17	7.9
Max	45.2	37.5	24.6	0.48	52.3
Average	37.3	19.2	12.5	0.28	23.3

Heritabilities

Table 3 shows the mean values, variance components and heritabilities of the variables of girth, height, and girth increments. It shows that the controlled variation components due to the blocks (B) and to the sub-blocks (S) were relatively low, whereas the uncontrolled variation of the error (E) was important. For girth, the latest measurements G64 and G71 exhibited the highest heritability ($h^2l = 0.42$). For height, H30 showed the highest heritability ($h^2l = 0.32$). Whereas girth increments exhibited low heritabilities, the girth increment P8 during the tapping period of 2007 exhibited the highest heritability of all the available growth traits ($h^2l = 0.45$).

Table 3: Mean values, variance components and heritabilities (h^2l) of girth, height measurements, and girth increments over 9 successive periods from 18 to 71 months, alternating dry and rainy seasons, and including 5 months of tapping (P8).

Var	Nb trees	Mean (cm)	% Clones G	% Blocks B	% Sub-blocks S	% Error E	h^2l	Age (month), or season
Girth								
G18	2379	10.04	24	6	4	67	**0.26**	18
G23	2416	10.65	21	8	5	66	**0.25**	23
G31	2472	15.94	18	9	6	67	**0.21**	31
G36	2477	17.03	13	11	11	65	**0.17**	36
G43	2467	22.20	11	12	9	68	**0.14**	43
G47	2477	22.60	15	16	7	62	**0.20**	47
G53	2479	28.49	22	11	8	59	**0.27**	53
G59	2478	30.86	27	3	9	62	**0.30**	59
G64	2486	36.12	41	0	2	57	**0.42**	64
G71	2486	37.19	40	0	3	57	**0.42**	71
Height								
H17	2485	412	21	2	6	71	**0.23**	17
H30	2479	515	28	6	8	59	**0.32**	30
H47	2477	602	17	25	4	55	**0.23**	47
H53	2411	782	23	9	9	59	**0.28**	53
H67	2451	919	17	10	13	61	**0.22**	67
Girth increment								
P1	2068	0.61	0	5	7	87	**0.00**	dry (5 months)
P2	2344	5.29	17	8	6	69	**0.20**	rainy (8 months)
P3	2076	1.09	2	20	37	41	**0.05**	dry (5 months)
P4	2317	5.17	3	39	15	43	**0.06**	rainy (7 months)
P5	1681	0.40	1	12	9	78	**0.02**	dry (4 months)
P6	2327	5.89	22	4	12	62	**0.26**	rainy (6 months)
P7	2324	2.37	8	22	9	61	**0.12**	dry (6 months)
P8	2333	5.26	36	11	9	44	**0.45**	tapping, rainy (5 months)
P9	2400	1.07	11	0	4	85	**0.11**	dry (7 months)
Cumulated girth increments								
R21	2296	16.35	9	31	14	46	**0.16**	rainy, 21 cumulated months
D20	1343	4.47	7	1	10	83	**0.07**	dry, 20 cumulated months
D27	1304	5.54	6	0	7	87	**0.06**	dry, 27 cumulated months

Table 4 shows the mean latex productions and their high heritabilities. The heritabilities of cumulated production of 9 tappings or more varied from 0.52 (P6S17cum) to 0.76 (P51, P5cum).

Table 5 shows the heritabilities of the 4 traits of Latex Diagnostic (Drc, Suc, Pi, Rsh) for each of the 5 series of measurements, and for the mean of the 5 series. The heritabilities of the mean LD were markedly increased and had a rather high level (from 0.30 to 0.59).

Table 6 shows the heritabilities of the 4 traits of each of the two series of Plugging Index measurements, and of the mean of the two series. These heritabilities varied between 0.38 and 0.57, which is rather high.

Table 4: Heritabilities of the main traits related to latex production (h^2l).

Var	Age (year)	Nb trees	Nb tappings	Mean g/t/t	h^2l
Measured productions					
P51	5	2346	10	5.61	0.76
P52	5	2347	11	4.88	0.66
P53	5	2346	10	7.10	0.68
P54	5	2328	10	5.82	0.59
P55	5	2347	10	10.87	0.58
(WT1)	5	1029	1	10.91	0.44
(WT2)	5	793	1	11.11	0.49
P61	6	2352	9-10	12.21	0.68
P6S1	6	1139	1	20.99	0.46
P6S2	6	1210	1	19.66	0.42
P6S3	6	1209	1	35.03	0.38
P6S4	6	1095	1	12.29	0.22
P6S5	6	1144	1	15.26	0.51
P6S6	6	1145	1	20.40	0.47
P6S7	6	1220	1	22.55	0.49
P6S8	6	2342	10	19.84	0.58
Cumulated productions					
P5cum	5	2326	51	7.03	0.76
P61	6	2352	9-10	12.21	0.68
P6S17cum	6	2224	3-4	20.72	0.52
P6S8	6	2342	10	19.84	0.58
P6cum	6	2196	22-24	16.82	0.65
Pcum	5-6	2101	73-75	10.08	0.75

Table 5: Heritabilities of Latex Diagnostic traits (h^2l).

h^2l	Drc	Suc	Pi	Rsh
LD1 (2007)	0.20	0.19	0.40	0.00
LD2 (2007)	0.37	0.18	0.43	0.19
LD3 (2007)	0.20	0.29	0.34	0.15
LD4 (2008)	0.27	0.19	0.44	0.26
LD5 (2008)	0.17	0.14	0.31	0.08
Mean LD	**0.54**	**0.44**	**0.59**	**0.30**

Table 6: Heritabilities of the traits of Plugging Index (h^2l).

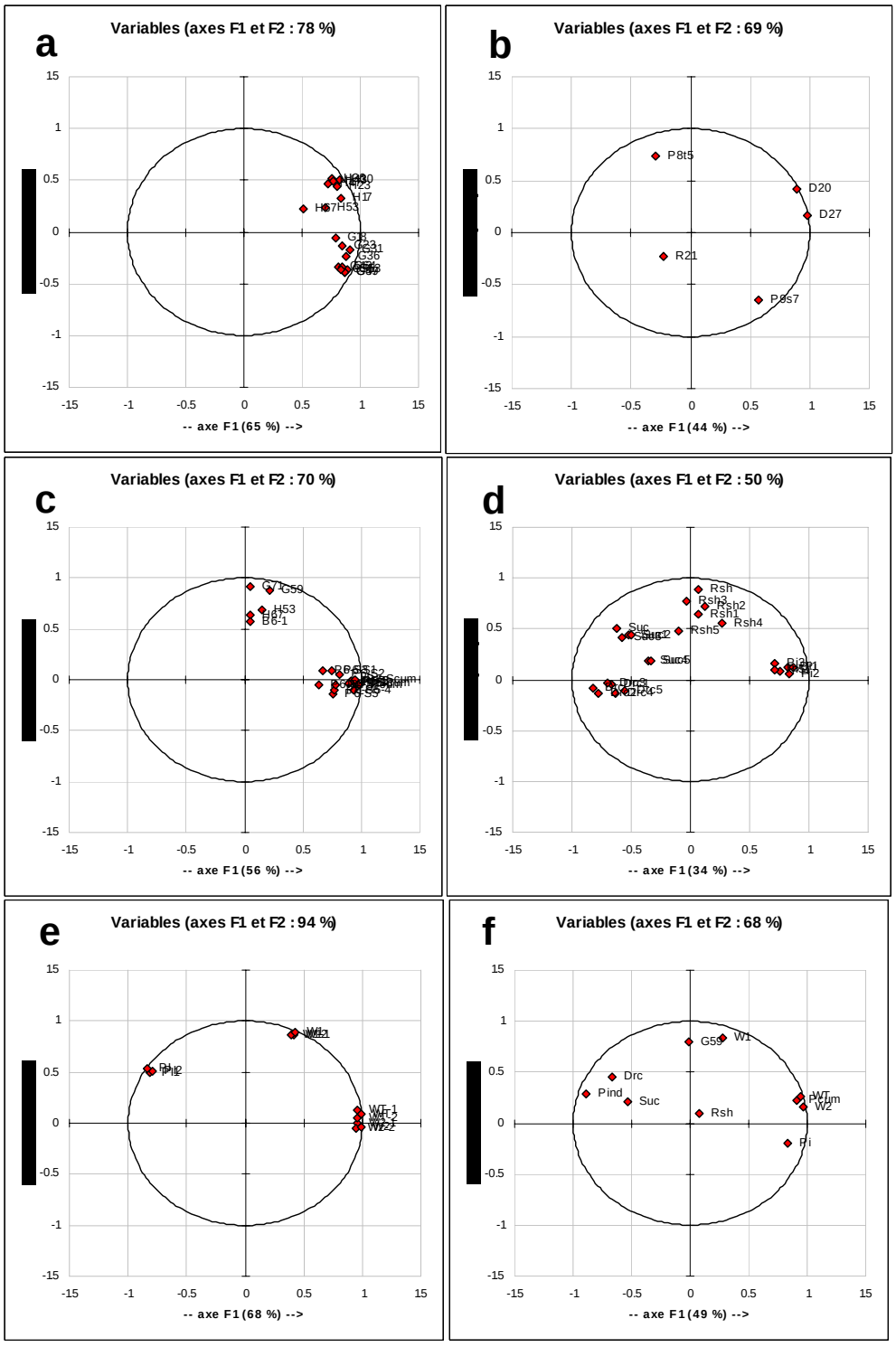
h^2l	W1	W2	WT	PI
First series	0.38	0.43	0.44	0.43
Second series	0.48	0.49	0.49	0.56
Mean series	**0.45**	**0.50**	**0.48**	**0.57**

Genetic correlations

Figure 1 presents 6 PCA graphs 1-2, noted a, b, c, d, e, f, that display the main trends of the correlations between the genetic values of the measured traits.

Figure 1 (a, b, c, d, e, f): Graphs 1-2 of Principal Components Analyses (PCA) featuring the relationships between measured traits.

a: Girth and height measurements, from the age of 18 months to 71 months
b: Five periods of girth increment
c: Growth and production measurements
d: Five series of Latex Diagnostic measurements
e: Two series of Plugging Index measurements
f: Synthetic view of the main sources of variation



On figure 1a, the PCA graph displays 78 % of the genetic variation of girth and height measurements. Axis F1 alone contains 65 % of the variation due to both the girth and height measurements. Although the two groups, girths and heights, are correlated, there are stronger correlations within each group. It was found that correlations between girth and height of similar ages, from 18 to 71 months of age, varied between 0.45 and 0.71.

Table 7 shows a negative correlation (r = - 0.20) between the growths in the rainy seasons (R21) and in the dry seasons (D20). It also shows that P8, the girth increment during the tapping period of the rainy season of 2007, was negatively correlated with P9, the girth increment of the following dry period (r = - 0.35). Moreover a strong negative correlation was also found between P8 and P5cum, the cumulated production of 2007 (r = - 0.54).

Table 7: Genetic correlations between different periods of girth increment. In bold : correlation coefficients significant for $\alpha = 0.05$).

Var	R21	D20	P8	P9
R21	1	**-0.20**	**0.23**	**0.17**
D20	**-0.20**	1	0.01	**0.18**
P8	**0.23**	0.01	1	**-0.35**
P9	**0.17**	**0.18**	**-0.35**	1

Figure 1b provides a visual representation of the correlations between those girth increments and mainly distributed over 3 directions, with D20, P9 and D27 associated to the axis F1, P8 and P9 in opposed positions on axis F2, and R21 associated to axis F3.

Figure 1c displays a visual representation of the relationships between girth at 59 and 71 months of age (G59, G71), height at 53 and 67 months of age (H53, H67), bark depth at 6 years of age(B61), and the different latex production traits measured at 5 and 6 years of age (P51, P52, P53, P54, P55, P5cum, P61, P6S1, P6S2, P6S3, P6S4, P6S5, P6S6, P6S7, P6S8, P6S17cum, P6S8, P6cum, and Pcum). Axis F1 was mainly associated to every production trait, and G59 was significantly correlated with this axis (r = 0.21).

Table 8 shows that girth G59 was correlated with the latex production traits P51, P52, P53, P55, P5cum, P61, P6S1, P6S2, P6S3, P6Scum, and Pcum. Therefore it can be considered to estimate and analyse the latex production components that are made independent from this girth.

Table 8: Genetic correlations between growth traits and latex production. In bold : correlation coefficients significant for $\alpha = 0.05$.

Var	G59	G71	H53	H67	B6-1
G59	1	**0.95**	**0.47**	**0.38**	**0.47**
G71	**0.95**	1	**0.48**	**0.43**	**0.47**
H53	**0.47**	**0.48**	1	**0.53**	**0.21**
H67	**0.38**	**0.43**	**0.53**	1	**0.14**
B61	**0.47**	**0.47**	**0.21**	**0.14**	1
P51	**0.21**	0.04	0.07	-0.04	0.09
P52	**0.20**	0.03	**0.14**	0.04	0.04
P53	**0.18**	0.01	0.13	0.02	0.03
P54	0.11	-0.05	0.09	-0.03	-0.01
P55	**0.20**	0.02	**0.14**	0.01	0.05
P5cum	**0.18**	0.00	0.11	0.00	0.04
P61	**0.15**	0.01	0.09	0.01	0.05
P6S1	**0.20**	0.12	**0.21**	0.11	0.07
P6S2	**0.16**	0.07	**0.21**	0.11	-0.02
P6S3	**0.18**	0.10	0.12	0.12	0.09
P6S4	0.11	0.00	0.01	0.02	-0.03
P6S5	0.05	-0.06	0.01	-0.07	0.01
P6S6	0.07	-0.05	0.06	0.01	-0.05
P6S7	0.12	-0.01	0.06	0.01	0.02
P6Scum	**0.19**	0.05	**0.14**	0.06	0.02
Pcum	**0.17**	0.00	0.11	0.01	0.04

Table 9 shows the evolution of the average values of Latex Diagnostic traits along time. A decreasing trend was observed for Suc, and a strong increase in Pi from series 4 to series 5 can be attributed to the stimulation.

Table 9: Average values of the traits of the 5 series of Latex Diagnostic carried out in 2007 (LD1, LD2, and LD3) and in 2008 before and after stimulation (LD4 and LD5).

	Nb trees	Drc	Suc	Pi	Rsh
LD1	793	34.1	22.5	11.6	0.32
LD2	791	38.6	21.5	14.6	0.29
LD3	788	39.2	18.3	14.2	0.26
LD4	783	41.8	14.0	10.3	0.28
LD5	785	37.4	15.3	22.1	0.29

Apart from those variations, PCA graph 1-2 of figure 1d shows that the 5 series of Latex Diagnostic measurements provided similar informations, which makes possible to focus on the average values of the 5 series. Those means had heritabilities higher than those of individual series. The graph also shows that axis F1 was strongly associated with both Pi and Drc which were negatively correlated between each other. Rsh was relatively independent from Pi and Drc, and it was mainly associated with axis F2. Suc was negatively correlated with Pi, and the correlation table of the PCA analysis showed that it was associated with the three axes F1, F2, F3. As a consequence, Latex Diagnostic information provided three main sources of variation.

Table 10 shows that the correlation coefficients between the traits of Latex Diagnostic of series 4 (before stimulation) and 5 (after stimulation) are lower than those linking series 1 and 2, series 2 and 3, and series 3 and 4. This indicates a change of behaviour of the clones after the stimulation, although it does not seem very important.

Table 10: Evolution of the correlations between the traits of Latex Diagnostic from one series to the following series. Series 1, 2, 3 carried out in 2007. Series 4 and 5 carried out in 2008, before and after stimulation.

1-2	Drc1	Suc1	Pi1	Rsh1
Drc2	**0.72**	**0.23**	**-0.58**	-0.02
Suc2	**0.15**	**0.65**	**-0.33**	0.10
Pi2	**-0.48**	**-0.29**	**0.85**	0.04
Rsh2	-0.06	0.07	0.10	**0.43**

3-4	Drc3	Suc3	Pi3	Rsh3
Drc4	**0.57**	**0.27**	**-0.36**	-0.02
Suc4	0.09	**0.44**	**-0.18**	-0.01
Pi4	**-0.47**	**-0.37**	**0.61**	-0.03
Rsh4	-0.12	-0.05	**0.17**	**0.49**

2-3	Drc2	Suc2	Pi2	Rsh2
Drc3	**0.66**	**0.20**	**-0.49**	-0.07
Suc3	**0.28**	**0.55**	**-0.38**	-0.02
Pi3	**-0.47**	**-0.28**	**0.73**	0.11
Rsh3	-0.01	0.09	-0.02	**0.56**

4-5	Drc4	Suc4	Pi4	Rsh4
Drc5	**0.40**	0.04	**-0.30**	-0.08
Suc5	0.09	**0.27**	-0.10	-0.12
Pi5	**-0.34**	**-0.26**	**0.59**	**0.21**
Rsh5	0.03	0.10	-0.02	**0.36**

The PCA graph 1-2 of figure 1e shows that the 2 series of Plugging Index measurements provide very similar informations. Therefore, one can focus on the average data of the 2 series. Axis F1 is closely associated to WT, W2, and Pind, with a negative correlation between on the one hand WT and W2, and on the other hand Pind, which is logically caused by the calculation mode of Pind. W1 (production of the first 5 minutes after tapping) is closely associated to axis F2. The traits of Plugging Index thereby provide 2 sources of variation.

The PCA graph 1-2 of figure 1f exhibits a correlative view of the traits that provide the main sources of variation. Table 11 shows the correlation coefficients between those traits and the first 4 axes of the PCA (F1, F2, F3, and F4). Axis F1 (the major source of variation) is associated with Pcum, Pi, Drc, W2, WT, Pind, and Suc. Suc is also associated to axes F3 and F4. Axis F2 is mainly associated with G59 and W1. Axis F3 is mainly associated with Rsh.

Table 11: Correlations between the main traits and the 4 axes of the Principal Component Analysis of the progenies data for those traits. In bold : some interesting correlation coefficients.

Var	**F1**	**F2**	**F3**	**F4**
G59	-0.01	**0.80**	-0.25	-0.28
Pcum	**0.91**	0.22	0.01	-0.01
Drc	**-0.66**	**0.46**	-0.09	-0.17
Suc	**-0.53**	0.21	**0.51**	**0.51**
Pi	**0.84**	-0.19	0.14	0.12
Rsh	0.08	0.10	**0.89**	-0.43
W1	0.28	**0.84**	0.07	0.25
W2	**0.96**	0.16	0.00	0.04
WT	**0.94**	0.26	0.01	0.08
Pind	**-0.88**	0.28	0.02	0.10

Table 12 confirms the strong correlation between production (Pcum, W2, WT) on the one hand, and Pi, Drc, and Pind on the other hand. It confirms the significant correlation of G59 with W1, but also with Pcum and WT at a low level. Therefore a small part of the production can be attributed to the vigour of the clones. We can notice a positive correlation between Suc and Drc, that can be explained by their negative correlation with production. We also observe a positive correlation between Suc and Rsh, but with a low level (r = 0.18).

Table 12: Genetic correlations between G59, Pcum, Drc, Suc, Pi, Rsh, W1, W2, WT, and Pind. In bold: correlation coefficients significant for $\alpha = 0.05$.

Var	G59	Pcum	Drc	Suc	Pi	Rsh	W1	W2	WT	Pind
G59	1	**0.17**	**0.31**	0.04	**-0.21**	-0.05	**0.44**	0.09	**0.14**	**0.15**
Pcum	**0.17**	1	**-0.49**	**-0.41**	**0.71**	0.10	**0.41**	**0.87**	**0.87**	**-0.73**
Drc	**0.31**	**-0.49**	1	**0.33**	**-0.59**	-0.04	0.12	**-0.53**	**-0.48**	**0.63**
Suc	0.04	**-0.41**	**0.33**	1	**-0.37**	**0.18**	0.04	**-0.43**	**-0.40**	**0.48**
Pi	**-0.21**	**0.71**	**-0.59**	**-0.37**	1	0.11	0.10	**0.73**	**0.70**	**-0.72**
Rsh	-0.05	0.10	-0.04	**0.18**	0.11	1	0.09	0.07	0.08	-0.05
W1	**0.44**	**0.41**	0.12	0.04	0.10	0.09	1	**0.39**	**0.50**	0.12
W2	0.09	**0.87**	**-0.53**	**-0.43**	**0.73**	0.07	**0.39**	1	**0.99**	**-0.85**
WT	**0.14**	**0.87**	**-0.48**	**-0.40**	**0.70**	0.08	**0.50**	**0.99**	1	**-0.78**
Pind	**0.15**	**-0.73**	**0.63**	**0.48**	**-0.72**	-0.05	0.12	**-0.85**	**-0.78**	1

Taking into account the contribution of the vigour of the clones to their production, the new variables Pcumi, Pii, Drci, W1i, W2i were estimated as independent from the girth G59 for the traits Pcum, Pi, Drc, W1, and W2 (covariance analysis). The experience of Latex Diagnostic as associated to the study of latex production has shown that Suc is negatively correlated with production because a high production consumes a large quantity of sucrose. Taking this knowledge into account, one has then estimated the component Sucip of sucrose as independent from the production trait Pcumi. The newly estimated traits exhibited a global variation that is featured by the PCA graph 1-2 of figure 2.

Figure 2: Graph1-2 of the Principal Components Analysis (PCA) featuring the relationships between G59, Pcumi, Pii, Drci, Pii, Rsh, W1i, W2i, Pindi, and Sucip.

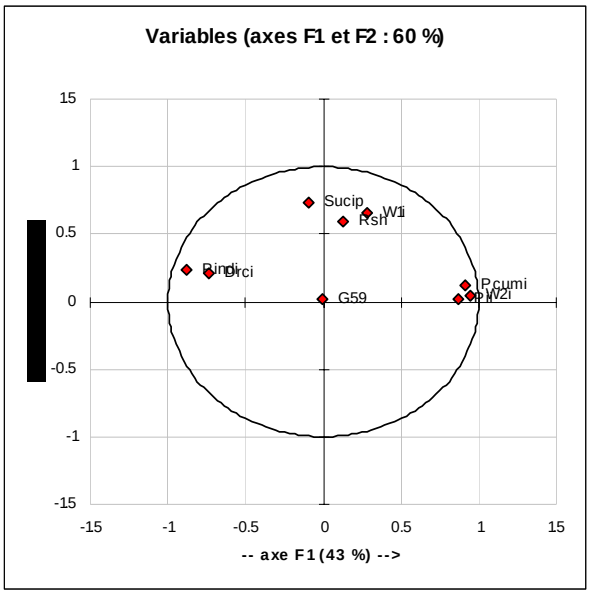


Table 13 shows the correlations between the newly estimated traits. Pcumi is highly correlated with Pii, Drci, W2i, Pindi, and also with W1i at a lower level (r = 0.35). W1i is positively correlated with Sucip at a rather low level (r = 0.19). And Sucip and Rsh were correlated at a higher level than Suc and Rsh (r = 0.25 as compared to r = 0.18 with the initial traits). Considering that Pcumi, W2i, Pindi, Drci and Pii were highly correlated between each other, the traits G59, Pcumi, W1i, Sucip, and Rsh appeared to be the traits least dependant from each other and making the most important part of the global genetic variation. The three traits W1i, Sucip and Rsh were positively corrrelated with F2 but they also differed from each other: W1i and Rsh were positively correlated with Sucip at a low level (r = 0.19 and r = 0.25 respectively), and they were not correlated between each other.

Table 13: Correlations between G59, Pcumi, W1i, W2i, Pindi, Drci, Sucip, Pii, and Rsh.

Var	G59	Pcumi	W1i	W2i	Pindi	Drci	Sucip	Pii	Rsh
G59	1	0.00	0.00	0.00	0.01	0.00	0.05	0.00	-0.05
Pcumi	0.00	1	**0.35**	**0.85**	**-0.75**	**-0.56**	0.00	**0.75**	0.11
W1i	0.00	**0.35**	1	**0.39**	0.06	-0.02	**0.19**	**0.22**	0.13
W2i	0.00	**0.85**	**0.39**	1	**-0.87**	**-0.59**	-0.09	**0.77**	0.08
Pindi	0.01	**-0.75**	0.06	**-0.87**	1	**0.62**	**0.17**	**-0.70**	-0.07
Drci	0.00	**-0.56**	-0.02	**-0.59**	**0.62**	1	0.10	**-0.56**	-0.02
Sucip	0.05	0.00	**0.19**	-0.09	**0.17**	0.10	1	-0.06	**0.25**
Pii	0.00	**0.75**	**0.22**	**0.77**	**-0.70**	**-0.56**	-0.06	1	0.10
Rsh	-0.05	0.11	0.13	0.08	-0.07	-0.02	**0.25**	0.10	1

Detection of QTLs

Table 14 shows that a major QTL was detected on the linkage group 3 around the position 60.2 for the 10 girth traits G18, G23, G31, G36, G43, G47, G53, G59, G64, G71, for the height trait H53, and also for the girth increment P9. It might be also the same QTL as that found for P7 and D27 in position 68.4. For this QTL predominantly associated to the girth of the trunk, maximum lod-score (MLS) tended to increase with the growth of the trees, with a value of 7.1 for G18 and values of 13.7 and 13.6 for G59 and G71 respectively.. The percentage of explanation (determination coefficient R^2) of the genetic variance of those traits varied from 16 % (G18) to 28 % (G59, G71). The molecular marker g3a312 is located exactly at the position 60.2 of the QTL named Hbg3a312 (Hb for *Hevea brasiliensis*).

For G64 and G71, a second QTL was detected on the linkage group 16 on positions 8.8 and 9.6 respectively, with MLS of 7.7 and 6.2 respectively. This is probably the same QTL as that found on position 7.8 for the girth increment during tapping P8, with the high MLS of 16.8 (R^2 = 34 %). For the height trait H30, a third QTL was detected on linkage group 8 at position 85.8, with MLS = 5.8. For the girth increment P7, 2 other QTLs were detected on linkage group 12 at positions 7.0 and 31.4, with MLS of 5.6 and 5.2 respectively. For P8, 2 other QTLs were detected on linkage group 5 at positions 94.4 and 70.1, with MLS of 5.3 and 4.7 respectively.

Table 15 shows that a major QTL was detected on the linkage group 16 around the position 5.8 for the latex production traits P51, P52, P53, P54, P55, P61, P6S1, P6S2, P6S5, P6S6, P6S7, P6S8, P5cum, P6S17cum, P6cum, and Pcum. The position varied from 4.0 to 7.8. MLS were as high as 35.5 (for P61) with R^2 = 59 %. The molecular marker nearest to the position 5.8 is g16a131 located on position 4.8. Therefore this QTL was named Hbg16a131.

For some production traits, more than one QTL were detected. One QTL was detected on group 3, at position 50.9 for P51, P53, P5cum, P6cum, and Pcum, at position 50.0 for P52, at position 68.4 for P6S7, at position 49.9 for P6S8. This QTL is very probably the same that was observed for girth. Its MLS varied, depending on the traits, between the significance level and 6.1.

Some other QTLs were observed, with MLS varying between the significance level and 6.2 :

- on group 11 at position 104.0 for P6S4
- on group 8 at position 25.2 for P51
- on group 2 at position 60.9 for P55, P6S17cum, and Pcum, at position 58.9 for P6S8, and at position 58.3 for P6cum
- on group 18 at position 93.9 for P6S8.

Table 14: Detection of QTLs associated to growth traits. Significance threshold: lod-score = 4.5 ($\alpha = 0.05$).

Var	QTL	Group	Position	Maximum lod-score	Significance	R^2
G18	1	3	60.2	7.1	**S**	16
G23	1	3	60.2	8.1	**S**	17
G31	1	3	60.2	8.3	**S**	18
G36	1	3	60.2	7.1	**S**	16
G43	1	3	60.2	10.4	**S**	22
G47	1	3	59.6	12.9	**S**	27
G53	1	3	59.6	12.0	**S**	25
G59	1	3	58.6	13.7	**S**	28
G64	1	3	60.2	11.7	**S**	24
G64	2	16	8.8	7.7	**S**	17
G71	1	3	59.6	13.6	**S**	28
G71	2	16	9.6	6.2	**S**	14
H17	1	3	64.2	3.9	ns	10
H23	1	8	85.8	4.4	ns	12
H30	1	8	85.8	5.8	**S**	15
H36	1	8	86.8	4.0	ns	11
H43	1	8	87.8	4.3	ns	11
H47	1	8	88.4	4.3	ns	11
H53	1	3	58.6	6.8	**S**	15
H67	1	8	90.4	4.4	ns	11
P1	1	3	73.4	3.1	ns	9
P2	1	3	16.6	3.4	ns	9
P3	1	8	104.2	3.3	ns	8
P4	1	2	19.4	2.6	ns	7
P5	1	6	70.1	2.9	ns	8
P6	1	6	47.8	2.8	ns	8
P7	1	3	68.4	5.8	**S**	15
P7	2	12	7.0	5.6	**S**	20
P7	3	12	31.4	5.2	**S**	13
P7	4	10	75.9	4.2	ns	12
P8	1	16	7.8	16.8	**S**	34
P8	2	5	94.4	5.3	**S**	14
P8	3	5	70.1	4.7	**S**	15
P9	1	3	58.6	4.6	**S**	11
R21	1	3	51.9	4.1	ns	10
D20	1	3	69.4	4.1	ns	11
D27	1	3	68.4	5.8	**S**	16
D27	2	17	16.0	4.1	ns	14
B61	1	3	17.9	3.8	ns	9

Table 15: Detection of QTLs associated to latex production traits. Significance threshold: lod-score = 4.5 (α = 0.05).

Var	QTL	Group	Position	Maximum lod-score	Significance	R^2
P51	1	16	5.8	20.3	**S**	40
	2	3	50.9	5.7	**S**	14
	3	8	25.2	4.7	**S**	11
P52	1	16	5.8	17.1	**S**	35
	2	3	50.0	5.5	**S**	13
P53	1	16	5.8	19.9	**S**	39
	2	3	50.9	4.6	**S**	12
P54	1	16	5.8	24.1	**S**	45
P55	1	16	5.8	16.9	**S**	34
	2	2	60.9	4.7	**S**	13
P61	1	16	5.8	35.5	**S**	59
P6S1	1	16	7.8	7.8	**S**	18
P6S2	1	16	4.8	9.8	**S**	20
P6S3	Nothing				ns	
P6S4	1	11	104.0	6.2	**S**	15
	2	16	4.0	4.4	ns	10
	3	1	14.3	4.1	ns	9
P6S5	1	16	6.8	15.1	**S**	32
P6S6	1	16	7.8	11.9	**S**	26
	2	2	64.9	4.1	ns	10
P6S7	1	16	4.0	11.2	**S**	24
	2	3	68.4	4.9	**S**	13
P6S8	1	16	5.8	17.4	**S**	35
	2	3	49.9	6.1	**S**	14
	3	2	58.9	4.6	**S**	14
	4	18	93.9	4.6	**S**	10
P5cum	1	16	5.8	22.3	**S**	43
	2	3	50.9	4.5	**S**	12
P6S17cum	1	16	6.8	15.0	**S**	32
	2	2	60.9	5.1	**S**	15
P6cum	1	16	5.8	24.0	**S**	45
	2	2	58.3	4.65	**S**	14
	3	3	50.9	4.3	ns	11
Pcum	1	16	5.8	24.7	**S**	46
	2	3	50.9	4.6	**S**	12
	3	2	60.9	4.4	ns	12

On table 16, the QTL Hbg16a131 was detected again for the traits Drc, Pi, W2, WT, and Pind with MLS higher than 20 and reaching 40.3 for Pind. It must be underlined that those traits are strongly correlated with latex production for which this major QTL was detected.

Hbg16a131 was also detected for Suc but with a slightly different position (4.8 instead of 5.8). Another QTL was detected for Suc on group 16 again but at position 25.9 different from that of Hbg16a131.

For Rsh, one QTL was detected on group 12 at position 90.1. For W1, 2 QTLs were detected, one on group3 at position 58.6 (may be the same QTL that was detected for girth), and the other on group 9 at position 13.1.

Table 16: Detection of QTLs associated to the traits of latex diagnostic and plugging index. Significance threshold: lod-score = 4.5 ($\alpha = 0.05$).

Var	QTL	Group	Position	Maximum lod-score	Significance	R^2
Drc	1	16	6.8	29.2	**S**	52
Suc	1	16	4.8	10.4	**S**	22
	2	16	25.9	8.1	**S**	20
Pi	1	16	5.8	29.5	**S**	52
	2	3	63.2	4.3	ns	10
Rsh	1	12	90.1	4.9	**S**	12
	2	12	76.4	4.2	ns	10
	3	12	70.3	4.1	ns	10
W1	1	3	58.6	8.6	**S**	19
	2	9	13.1	4.6	**S**	14
	3	8	21.2	4.2	ns	9
	4	8	49.2	4.2	ns	11
W2	1	16	5.8	27.0	**S**	50
WT	1	16	5.8	22.0	**S**	43
Pind	1	16	5.8	40.3	**S**	64

QTL detection was also applied to the traits P5cumi, P61i, P6S17cumi, Pcumi, and Pii that were re-estimated for being independent from the girth G59 (table 17). In this case, for P5cumi, P61i, and P6S17cumi, Hbg16a131 was detected with higher MLS than for initial traits.

For Pii, MLS of Hbg16a131 was lower for Pii than for Pi. But another QTL was detected on group 3 at position 69.4.

Table 17: Detection of QTLs associated to latex production traits from which the share predicted by the girth G59 has been deducted. Significance threshold: lod-score = 4.5 ($\alpha = 0.05$).

Var	QTL	Group	Position	Maximum lod-score	Significance	R^2
P5cumi	1	16	5.8	27.8	**S**	50
P61i	1	16	5.8	43.8	**S**	67
P6S17cumi	1	16	6.8	18.3	**S**	37
Pcumi	1	16	5.8	33.1	**S**	57
W1i	1	9	13.1	5.8	**S**	18
	2	8	21.2	4.1	ns	9
Pii	1	16	4.8	26.9	**S**	47
	2	3	69.4	4.6	**S**	12
Drci	1	16	6.8	25.4	**S**	47

QTL detection was also applied to the trait Sucip that was re-estimated from Suc so as to become independent from latex production Pcum (table 18). In this case the 2 QTLs that were detected on Suc did not appear again, and no other possible QTL was found significant.

Table 18: Detection of QTLs associated to the Sucip component, independently from the latex production trait Pcumi. Significance threshold: lod-score = 4.5 ($\alpha = 0.05$).

Var	QTL	Group	Position	Maximum lod-score	Significance	R^2
Sucip	1	16	42.6	3.9	ns	10
Sucip	2	7	43.0	3.6	ns	9
Sucip	3	5	104.4	3.5	ns	9
Sucip	4	4	61.3	3.2	ns	8

The 2 markers of the major QTLs Hbg3a312 and Hbg16a131 provided the allelic segregations ef x eg and ab x cd respectively, with the following classes for the progenies :

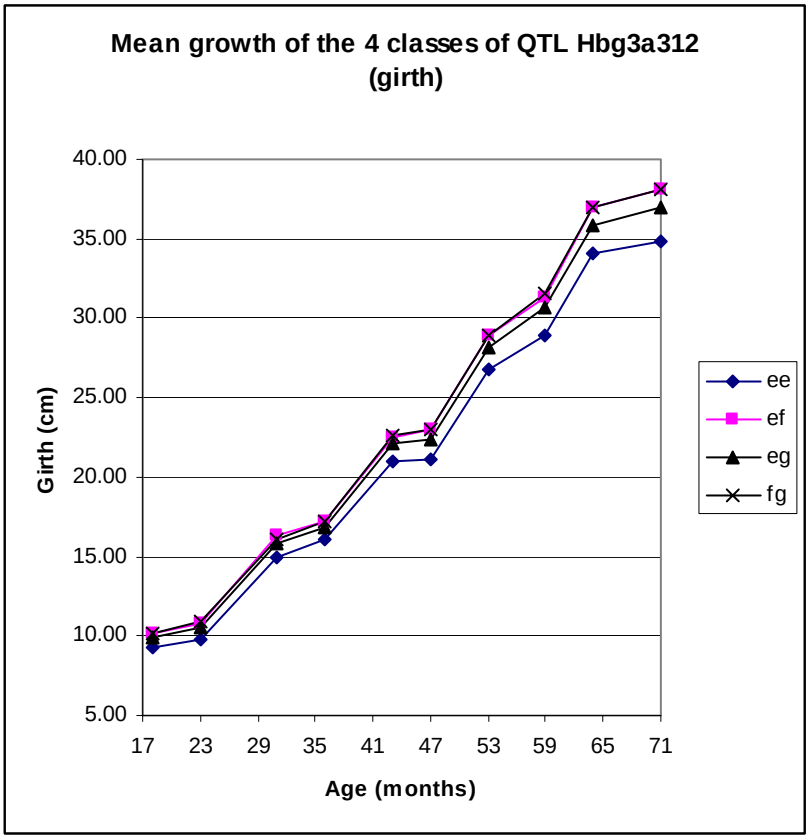
- ee, ef, eg, and fg for Hbg3a312
- ac, ad, bc, and bd for Hbg16a131

Tables 19 and 20 present the mean values of the traits for each class, so allowing to appreciate the agricultural differences between those classes. In table 19, for all the traits excepted one (D20), the class “ee” was always significantly lower than the three other classes. For G47, G59; G64, and G71, the class “eg” was lower than “ef” and “fg” but higher than the class “ee”. For D20, only the class “fg” was significantly higher than the class “ee”. Figure 3 shows the evolution of girth along time for the 4 classes, illustrating the inferiority of the class “ee” for radial growth of the trunk (girth).

Table 19: Mean girths and cumulated girth increments of the 4 classes defined by Hbg3a312. The female parent RRIM600 belongs to class “ef”; the male parent PB217 belongs to class “eg”. Statistical significance for $\alpha = 0.05$.

Girth (cm)				
Age (months)	ee	ef	eg	Fg
18	9.27 b	10.22 a	9.95 a	10.16 a
23	9.80 b	10.82 a	10.54 a	10.86 a
31	14.94 b	16.27 a	15.79 a	16.11 a
36	16.11 b	17.27 a	16.89 a	17.26 a
43	20.94 b	22.54 a	22.12 a	22.62 a
47	21.07 c	22.98 a	22.37 b	23.02 a
53	26.77 b	28.98 a	28.22 a	28.96 a
59	28.90 c	31.38 a	30.63 b	31.58 a
64	34.02 c	36.99 a	35.88 b	36.95 a
71	34.87 c	38.09 a	36.99 b	38.13 a
Cumulated girth increments (cm)				
R21	15.66 b	16.72 a	16.34 a	16.55 a
D20	3.98 b	4.44 ab	4.35 ab	4.86 a
D27	4.83 b	5.54 a	5.46 a	6.04 a

Figure 3: Evolution of the mean girths of the 4 classes defined by the marker Hbg3a312 associated to the girth QTL.



In table 20, for the traits P51, P52, P53, P54, P55, P5cum, P61, P6S17cum, P6S8, P6cum and Pcum, the class “bd” was always higher than the classes “ad” and “bc”, and those two classes were always higher than the class “ac”. For the traits P6S1, P6S2, P6S3, P6S4, P6S5, P6S6, and P6S7 that were measured on only one tapping and on a lower number of trees, the significance of the differences were not so clearcut. But the classes “ad” and “bc” were never different from each other, and the class “bd” was always higher than the class “ac”. For Pcum, the class “bd” exhibited a yield level of 149 % as compared to the mean latex production, whereas the class “ac” exhibited a level of 66 %.

Table 20: Mean latex production, in grams per tree per tapping, and indexes related to the mean productions of the 4 classes defined by Hbg16a131. Each of the two parents was represented twice in the trial (2 "clones" per parent). Statistical significance for α = 0.05.

	Gram / tree / tapping					
Classes	ab	**ac**	**ad**	**bc**	**bd**	cd
Nb clones	2	52	51	51	42	2
	RRIM600					PB217
P51	4.75	3.34 c	4.93 b	6.01 b	8.76 a	6.57
P52	3.74	3.33 c	4.45 b	5.27 b	6.88 a	5.76
P53	5.56	4.89 c	6.48 b	7.43 b	10.26 a	6.81
P54	5.33	3.70 c	5.23 b	6.01 b	8.96 a	5.36
P55	10.87	7.47 c	10.14 b	11.96 b	14.67 a	10.17
P61	9.92	6.77 c	9.60 b	11.07 b	23.59 a	12.69
P6S1	18.15	16.94 b	19.32 b	21.30 b	28.16 a	13.61
P6S2	20.25	14.91 c	18.24 bc	20.33 b	26.25 a	22.85
P6S3	35.47	30.50 b	33.17 b	35.87 ab	41.55 a	41.63
P6S4	11.92	9.82 c	11.30 bc	13.10 ab	15.51 a	14.16
P6S5	15.32	8.19 c	13.46 b	15.98 b	25.64 a	8.87
P6S6	12.05	14.69 c	19.38 b	20.79 b	28.77 a	17.37
P6S7	13.92	14.37 c	19.63 b	25.66 ab	32.99 a	20.22
P6S8	17.87	13.88 c	18.21 b	21.92 b	26.89 a	17.31
P5cum	6.21	4.65 c	6.42 b	7.52 b	10.18 a	7.16
P6S17cum	26.87	21.94 c	27.67 b	30.77 b	40.40 a	24.29
P6cum	15.88	12.76 c	17.19 b	19.41 b	28.32 a	16.05
Pcum	**10.18**	**7.38 c**	**10.20 b**	**11.60 b**	**16.36 a**	**9.92**

	Index / mean g/t/t					
Classes	ab	**ac**	**ad**	**bc**	**bd**	cd
Nb clones	2	52	51	51	42	2
	RRIM600					PB217
P51	85	59	88	107	156	117
P52	77	68	91	108	141	118
P53	78	69	91	105	145	96
P54	92	64	90	103	154	92
P55	100	69	93	110	135	94
P61	81	55	79	91	193	104
P6S1	86	81	92	101	134	65
P6S2	103	76	93	103	134	116
P6S3	101	87	95	102	119	119
P6S4	97	80	92	107	126	115
P6S5	100	54	88	105	168	58
P6S6	59	72	95	102	141	85
P6S7	62	64	87	114	146	90
P6S8	90	70	92	110	136	87
P5cum	88	66	91	107	145	102
P6S17cum	91	74	93	104	136	82
P6cum	84	67	91	103	150	85
Pcum	**92**	**66**	**92**	**104**	**147**	**89**

Discussion

Heritabilities

A high broad sense heritability was found for latex production (h^2l = 0.75 for the cumulated production Pcum), although genetic variability was limited to that of only one full-sib family. Heritability was also rather high for the two components of the production W1 and W2, for Pi in each series of latex diagnostic, and also for the mean Drc and Suc of the 5 series of latex diagnostic. For girth and height, heritability is more or less of the same level and generally lower than 0.30. For girth, the highest observed heritability was h^2l = 0.42 for G64 and G71. For height, the highest heritability was h^2l = 0.32.

The high heritability of the girth increment during tapping (P8) must be noticed. In fact, latex cells under tapping behave as a new physiological sink and induce an important reduction in growth fastness of the trees due to their uptake of photosynthetic assimilates at the detriment of growth. A strong negative correlation was observed between P8 and the corresponding latex production P5cum, suggesting a link between the high heritability of P8 and the high heritability of latex production.

Of course heritability depends on the specific conditions of the trial. But a high heritability means large genetic differences between the clones, and therefore better chances to find differences between segregating classes and to detect QTLs.

Genetic correlations

A high positive correlation was found between girth and height traits.

A negative correlation was found between the cumulated girth increments of the rainy season (R21) and of the dry season (D20). Although growth in the dry seasons is much slower than in the rainy seasons, and although the correlation coefficient is rather low (r = - 0.20), this could mean that the clones most fitted to grow in the dry season would « pay » this adaptation by some associated physiological constraints that would reduce their growth ability in the rainy season.

A strong correlation was shown between latex production (Pcum, W2, WT), the two traits Pi and Drc of the latex diagnostic, plugging index, and also with Suc. These results confirm what was observed in most physiological studies on varied sets of clones, and they can be interpreted in the light of the knowledge on rubber tree physiology (d'Auzac et al., 1997).

Tapping, as a wounding stress, induces biosynthesis of ethylene that generates the activation of the metabolism of bark and latex cells, notably the energetic metabolism, as indicated by a high level of Pi. Another important effect of ethylene is to increase the influx of water from xylem towards the latex cells, as indicated by the decrease in Drc level. This water influx generates the « dilution effect »: latex becomes less viscous and therefore it can flow during a longer period after tapping until coagulation, which results in a higher production. Considering the traits of Plugging Index, W1 is mainly dependant on the turgor pressure at the onset of tapping, whereas W2 is directly related to Drc and to the duration of latex flow. The clones most

responsive to tapping stress have a high level of W2 and a low level of Plugging Index. After coagulation, the activated metabolism regenerates a high amount of latex and rubber, so requiring a high consumption of sucrose for the production of energy and of the rubber chains. This explains the negative correlation between sucrose and latex production. A low sucrose content is partly a consequence of a high latex production. The correlations observed in this study confirm that those clones behave according to the classical physiological pattern, and thereby it can be waited that the results of this study could be confirmed and extended in other conditions.

Latex production and correlated traits data were modified for analyzing only the components independent from the girth of the trunk. In the same way, Suc data were made independent from latex production. The table of correlations between the new traits called Pcumi, W1i, W2i Pindi, Drci, Sucip, Pii, and Rsh confirms the strong link between Pcumi, Pii, Drci, W2i, and Pindi, and the independance of Rsh from latex production. A positive correlation is also observed between Sucip and W1i on the one hand ($r = 0.19$), and between Sucip and Rsh on the other hand ($r = 0.25$), but no interpretation was found for those relationships.

QTL Detection

A major QTL (Hbg16a131) directly associated to latex production was discovered, explaining $R^2 = 57$ % of the genetic variance of the cumulated latex production Pcumi. This QTL was also associated to the other correlated traits such as Pi ($R^2 = 52$ %), Drc ($R^2 = 52$ %), Pind ($R^2 = 64$ %). The four genotypic classes defined by the marker and issued from the mendelian segregation “ab x cd” were significantly different (“ac” < “ad” and “bc” < “bd”), and ranged from 66 % to 147 % of the average gram per tree per tapping of the cumulated production Pcumi.

Therefore there is one genetic factor (one gene or one cluster of genes) located on group 16 and position 5.8, able to determine a large part of the genetic variance of latex production. This can be related to the fact that latex production is generated by the abiotic stress of wounding due to tapping. It was already reported that major QTLs can be found more frequently for traits related to abiotic traits than for other types of complex traits (Mackill et al., 2006). In the conditions of this study, this QTL determined half of the genetic variance. The fact that the correlations between the measured traits fit to the normal physiological functioning of latex production suggests that ecological conditions may have a rather limited influence on this result, whereas the identity of the alleles involved is probably more important. One should find a different magnitude, lower or higher, with another genetic population.

A second important QTL (Hbg3a312), associated to the girth of the trunk, was detected. Its significance level tended to increase with the age of the trees, and its maximum lod-score was higher than 10 after 43 months of age (3.5 years). Depending on the age of the trees, it explained from 16 % to 28 % of the genetic variance of the girth. The 3 alleles of the nearest marker g3a312 segregated according to the pattern “ef x eg” and generated the 4 classes “ee”, “ef”, “eg”, and “fg”. For all the girth measurements, the class “ee” was found smaller than the three other classes. For the girth G59 measured at 5 years of age, just before initial opening, the average girth of the class “ee” was of 93 % of the average level of the 3 other classes. Although this effect is moderate, this QTL might be used in MAS too.

By contrast, no significant QTL was found for Sucip, the sucrose component estimated as independent from the production. Availability of sucrose in the latex cells is a major condition for the intensification of latex production by tapping and stimulation. This result suggests that sucrose content might not be determined by any single gene or cluster with significant effect and should be selected by classical quantitative genetics methods.

One QTL with a low effect was found for Rsh. Two QTLs were found for W1, the initial production measured during the first 5 minutes of latex flow. But this component of the production is not considered as very important for the selection of high-yielding clones.

So far, those results were found very stable and reproducible along time. But production was obtained only with a low-intensive tapping system. Although production was increased (nearly doubled) by stimulation, the correlations between the clones did not seem to be much modified after stimulation, the QTL Hbg16a131 maintained its important effect just after the stimulation, and no new QTL was detected after this stimulation.

The 2 major QTLs that were found associated to latex production and growth have estimated effects (R^2) much more important than that of other QTLs found for the same traits. Compilations in other species consistently reveal extremely skewed distributions of QTL effects, with few loci causing most of the genetic variation and resulting in a typical L-shaped distribution of QTL effects (Bost et al., 2001). This aspect, still in discussion, suggests that the observed effects of major QTLs might be inflated. Confirmation in other genetic and ecological conditions of the importance of the 2 QTLs detected by this study would therefore be useful.

Markers-Assisted Selection

The idea that the QTL Hbg16a131 would lead to the selection of all the genotypes belonging to the best genotypic class “bd” would ignore the share of genetic variance not explained by the QTL but also representing about half of this variance. Some performant clones also exist out of this best class and should be selected. Genotyping the QTL for all the candidates to selection should be used for enhancing the accuracy of the estimation of their genetic values, resulting in increased selection efficiency.

In quantitative genetics, genetic values are based on the linear combination of the different genetic contributions, for example family and individual contributions (Gnagne et al., 1997), with coefficients based on the estimation of variances (family, individual, and environmental variances). Accuracy of variance estimations is highly dependant on the number of data available for the estimation (number of plants). As a consequence, family variance is almost always more accurately estimated than individual variance. This principle of combined “family x individual” selection can be applied to MAS by considering the classes of segregation of one QTL as “families”.

In rubber tree, clonal selection is made of 3 successive steps, 1) Seedling Evaluation Trial (SET), 2) Small Scale Clonal Trial (SSCT), and 3) Large Scale Clonal Trial (LSCT). In SSCT and LSCT, each genotype is replicated in a small and in a large number of budded trees respectively, which makes possible an accurate control of

environmental variation. Moreover, selection in SSCT and LSCT is carried out on large areas and over many years. By contrast, SET is used for screening all the seedlings issued from hand pollination with poor information, which leads to low investment brought to this early stage, only to fastly reduce the number of candidates with low accuracy. There is generally no attempt to bud 2 or 3 copies of each genotype in order to make possible the distinction between genetic and environmental variances.

The important effect of the QTL Hbg16a131 can justify more investment on initial selection for latex production in rubber tree. Genotyping the QTL would be required for the candidates to selection, with ideally 2 or 3 markers neighbouring both sides of the QTL. When applied to varied families with more than 4 alleles at the QTL, this information would allow the classification of the genotypes into a number of segregation classes of the QTL, the deduction of their mean latex production, and the estimation of the variance between those classes. Moreover, multiplying each genotype in 2 or 3 budded copies as soon as at the first selection stage would generate added value by making possible the estimation of the environmental variance. In a simpler option, with assumption on the importance of environmental variance, selection could be applied to seedlings (one tree per genotype) with QTL genotyping limited to the only marker g16a131 located nearest to the position of the QTL. Whatsoever the implementation of MAS in SET would allow the assessment of new alleles at the QTL and confirm the validity of the results of this research. It appears very promising for increasing the efficiency of rubber clonal selection.

Acknowledgements

This research (Genmap project) is part of the project “Towards the improvement of rubber tree productivity” developed in Thailand by Kasetsart University, Rrit-Doa, Prince of Songkhla University, and Cirad, under the cover of the Hevea Research Platform in Partnership (HRPP). We wish to thank the “French-Thai Committee for Research and Higher Education”, as well as Agropolis for funding this research. We also wish to thank the technical staff of the breeding team of Chachoengsao Rubber Research Center (Crrc, Rrit-Doa) for the collection of the very large quantity of data that were necessary for achieving the results presented here.

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